

UK Patent Application (19) GB (11) 2 319 838 (13) A

(43) Date of A Publication 03.06.1998

(21) Application No 9720959.7

(22) Date of Filing 21.10.1997

(30) Priority Data

(31) 08739396

(32) 31.10.1996

(33) US

(71) Applicant(s)
Hewlett-Packard Company

(Incorporated in USA - California)

3000 Hanover Street, Palo Alto, California 94304,
United States of America

(72) Inventor(s)
Carol T Schembri

(74) Agent and/or Address for Service
Williams, Powell & Associates
4 St Paul's Churchyard, LONDON, EC4M 8AY,
United Kingdom

(51) INT CL⁵
C12Q 1/68, C07K 1/04

(52) UK CL (Edition P)
G1B BAG B523 B524 B525

(56) Documents Cited
WO 96/18903 A WO 95/33846 A WO 94/05394 A

(58) Field of Search
UK CL (Edition O) G1B BAG
INT CL⁶ C07K 1/04 17/00 17/02 17/06, C12Q 1/68
ONLINE: WPI, CLAIMS

(54) Producing a chemical (biochip) array

(57) A process of constructing an array of chemical moieties comprises the steps of: forming multiple discrete physical entities (tiles) (9) from a substantially planar material having one or more species of chemical moiety 11 attached thereto; and picking and placing the entities (tiles) stably on a support 13 at spatially distinct ascertainable locations to form an array of chemical moieties. The formed array includes at least two species of chemical moiety and preferably from about 50 to about 1000 species. The tiles are typically biochips bearing, for example, nucleic acids. Linkers may be employed to attach moieties to the tiles.

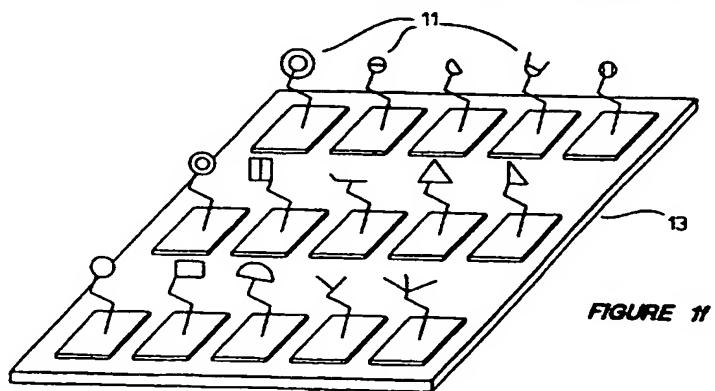


FIGURE 11

GB 2 319 838 A

BEST AVAILABLE COPY

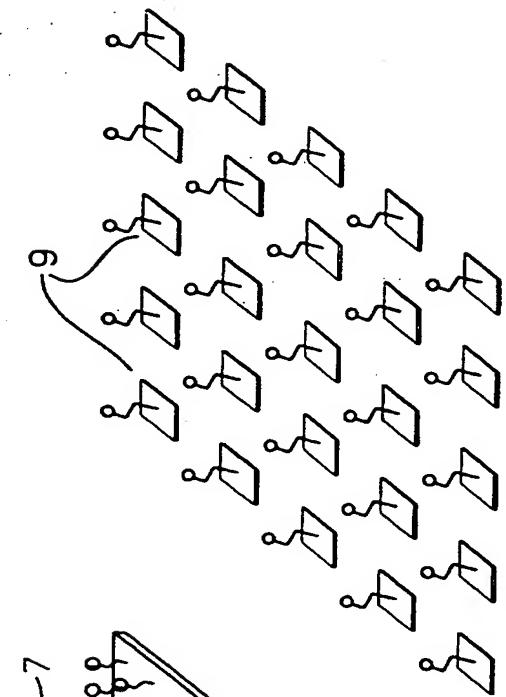


FIGURE 1d

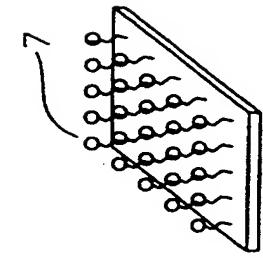


FIGURE 1e

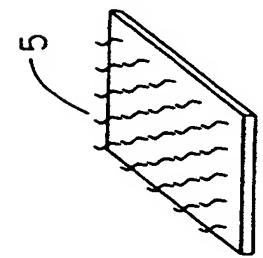


FIGURE 1f

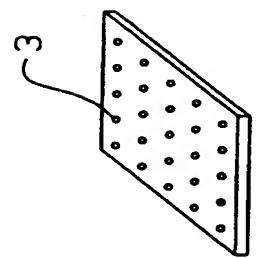


FIGURE 1g

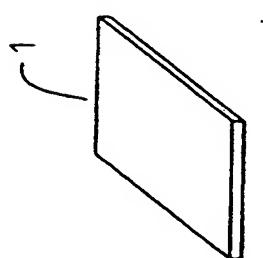


FIGURE 1h

FIGURE 1e

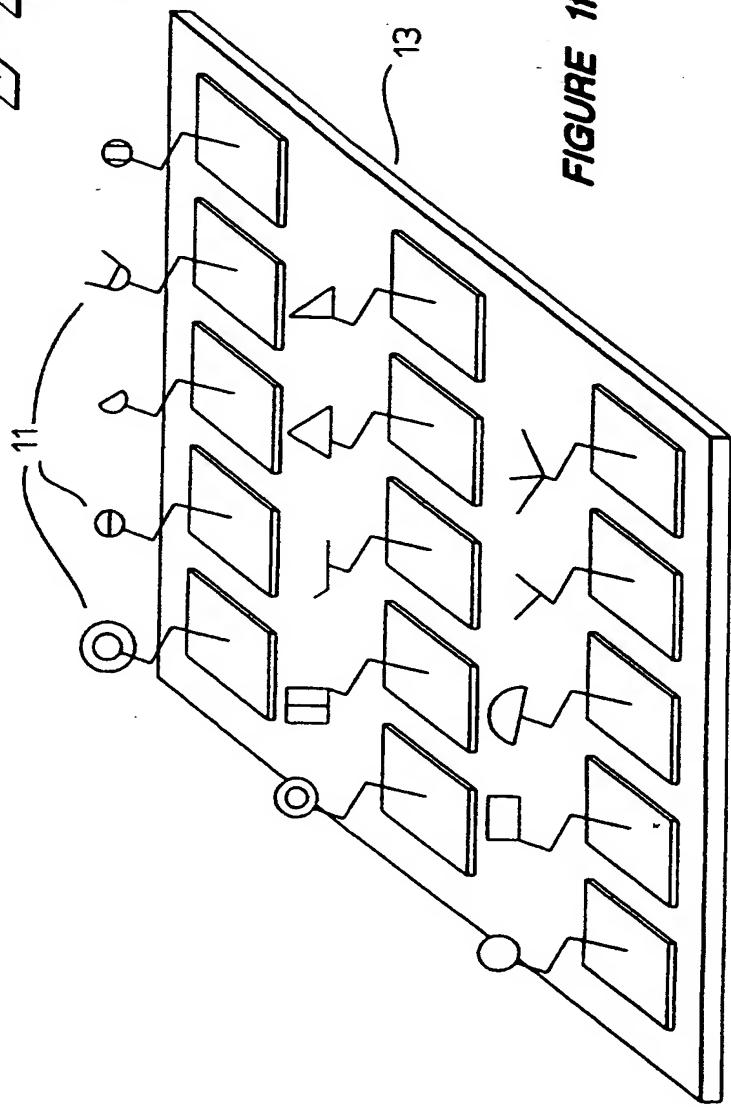


FIGURE 1f

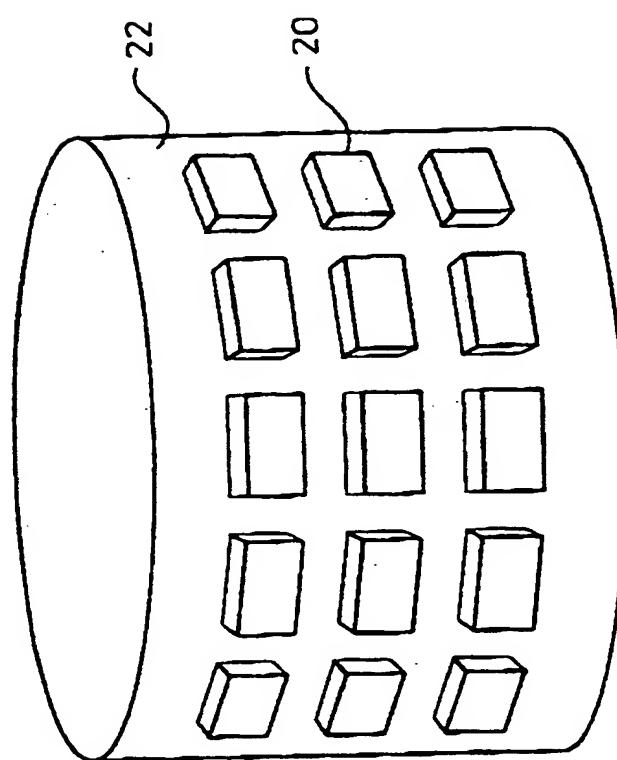


FIGURE 2

2319838

PRODUCING A CHEMICAL ARRAY

This invention relates generally to a process of forming an array of bioorganic molecular probes on a support, and to an array formed by such a process, e.g. a tiling process.

Arrays of immobilized probes are currently being developed for use in assays to detect and identify components in biological samples and for screening molecular libraries. The ability to screen for multiple species of molecules in a single assay test is particularly valuable for purposes of drug discovery and clinical genetics. Accordingly, array manufacturing technologies have been developed to permit a large number of different probes to be incorporated into an array at separate and known locations (see, e.g., Fodor et al., *Science* 251, 767-773 (1991); Southern et al., *Nucleic Acids Research* 22: 1368-1373 (1994); US Patent No. 5,510,270; US Patent No. 5,474,796; US Patent No. 5,429,807; and US Patent No. 5,472,672). In these prior art methods, the probe molecules are synthesized *in situ* on a solid support surface at predetermined locations.

There are disadvantages to forming an array in this manner when the array is comprised of biopolymers such as oligonucleotides. The *in situ* method currently used for the commercial manufacture of oligonucleotide arrays is not well suited for the efficient production of arrays of long chain polymer probes because of the number of cycles of elongation required and the variable efficiency of each attachment step. For example, in order to synthesize n species of oligonucleotide having m variable positions, $n \times m$ elongation cycles are required. Although solutions have been devised to shorten the time of the overall process by segregating groups of sites for the

simultaneous addition of a given nucleotide (see, e.g., Frank et al., Nucleic Acids Res. 11, 4365-4377 (1983); US Patent No. 5,510, 270), manufacturing an array in this way is inefficient, particularly when the desired array is intended to include hundreds of different probe sequences and probe lengths in excess of 30 nucleotides. Each attachment step requires a finite time for covalent bond formation, and each is associated with a failure rate of between 2% and 15%. The problems with probe fidelity necessitate a high level of probe redundancy, and it is only after the entire array is formed that defects are discoverable.

Additionally, if reagents are dispensed as microdroplets, precautions must be taken to avoid intermixing of chemical moieties in neighboring droplets either by precisely depositing the droplet at its designated attachment site or by forming a pattern of differential polarities on the attachment surface to constrain the droplets to their designated attachment regions by means of surface tension.

What is needed is a process for forming an array with probes of known high fidelity in which the chemistry of attachment can be independently optimized for each different probe in the array, the density of each type of attached probe can be quantitatively assessed prior to array formation and probes not meeting specifications can be corrected or discarded prior to array assembly. The number of overall steps required to form the array is reduced in this process compared to prior art methods, and the total number of tests required to ensure the quality of manufactured arrays is equal to the number of probes in the array, not the number of arrays produced.

To address the above-mentioned need in the art, the invention disclosed and claimed herein provides a process for constructing an array of

chemical moieties by forming multiple tiles comprised of a substantially planar material, each tile having attached to it at least one species of chemical moiety, and picking and placing each tile stably on a support at a separate and distinct spatial location to form an array of chemical moieties. The array formed by this process includes at least two species of chemical moiety and preferably from about 50 to about 1000 species of chemical moiety. The chemical moieties are preferably bioorganic molecular probes, most preferably nucleic acids, proteins, polysaccharides, and lipids.

An objective of this invention is to increase the speed and reproducibility of the array manufacturing process by attaching presynthesized probes to discrete physical entities which can be moved robotically to separate predetermined locations on a support to create the array. The attachment step is preferably carried out in a batchwise manner to optimize the linker chemistry and attachment density of each type of probe in the array independently of the assembly of the array.

A related objective is to provide means for optimizing the linker chemistry and attachment density of each type of probe in the array independently of the assembly of the array.

Yet another objective of this invention is to streamline the quality control procedure and lower the cost of producing an array with a high degree of chemical complexity. The fidelity of synthesis of each probe type in the array and its density of attachment can be verified prior to tiling the array. The entire surface of a discrete physical entity can be tested prior to subdividing it into hundreds of thousands of tiles for placement into hundreds of thousands of arrays, thereby permitting the early detection and correction of manufacturing problems.

Figure 1 illustrates schematically the steps involved in the process of forming an array of this invention.

Figure 2 illustrates an embodiment of the array in which the tiles are cylindrically arranged on a support surface.

Before the invention is described in detail, it is to be understood that this invention is not limited to the particular component parts or process steps of the methods described, as such parts and methods may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly indicates otherwise.

In this specification and in the claims which follow, reference will be made to a number of terms which are used as defined below.

An "array" is an arrangement of objects in space in which each object occupies a separate predetermined spatial position. Each of the objects in the array of this invention comprise one or more species of chemical moiety attached to a "discrete physical entity", such that the physical location of each species is known or ascertainable. A "discrete physical entity" is a unit of substantially planar material (e.g., a solid material, a membrane, a gel or a combination of materials) that can be handled and still maintain its identity, and can be subdivided into "tiles" for recombining in various ways to form a physical array. Preferably, the tiles will have regular geometric shapes, e.g., a sector of a circle, a rectangle, and the like, with radial or linear dimensions of about 100 μ m to about 10mm, most preferably about 250 μ m

to about 1000 μ m. The subdivision of the entity into tiles can be made either before or after attachment of the chemical moiety, and by any suitable method for cutting the entity, e.g., with a dicing saw. These methods are well-known in the art of semiconductor chip manufacture and can be optimized by one skilled in the art for the particular material selected for use in this invention.

A "support" is a surface or structure for the attachment of tiles. The "support" may be of any desired shape and size and can be fabricated from a variety of materials. The support material can be treated for biocompatibility (i.e., to protect biological samples and probes from undesired structure or activity changes upon contact with the support surface) and to reduce non-specific binding of biological materials to the support. These procedures are well-known in the art (see, e.g., Schöneich et al, Anal. Chem. 65: 67-84R (1993)). The tiles can be attached to the support by means of an adhesive, by insertion into a pocket or channel formed in the support, or by any other means that will provide a stable and secure spatial arrangement.

"Tiling" is the process of forming an array by picking and placing individual tiles comprising single or multiple species of chemical moieties on a support in a fixed spatial pattern.

A "chemical moiety" is an organic or inorganic molecule that is preformed at the time of attachment to a discrete physical moiety, in distinction to an organic molecule that is synthesized *in situ* on an array surface. The preferred mode of attachment is by covalent bonding, although noncovalent means of attachment or immobilization might be appropriate depending on the particular type of chemical moiety that is used. If desired, a "chemical moiety" can be covalently modified by the addition or removal of groups after the moiety is attached to a physically distinct entity.

The chemical moieties of this invention are preferably "bioorganic molecules" of natural or synthetic origin, are capable of synthesis or replication by chemical, biochemical or molecular biological methods, and are capable of interacting with biological systems, e.g., cell receptors, immune system components, growth factors, components of the extracellular matrix, DNA and RNA, and the like. The preferred bioorganic molecules for use in the arrays of this invention are "molecular probes" selected from nucleic acids (or portions thereof), proteins (or portions thereof), polysaccharides (or portions thereof), and lipids (or portions thereof), for example, oligonucleotides, peptides, oligosaccharides or lipid groups that are capable of use in molecular recognition and affinity-based binding assays (e.g., antigen-antibody, receptor-ligand, nucleic acid-protein, nucleic acid-nucleic acid, and the like). An array may contain different families of bioorganic molecule, e.g., proteins and nucleic acids, but typically will contain two or more species of the same family of molecule, e.g., two or more sequences of oligonucleotide, two or more protein antigens, two or more chemically distinct small organic molecules, and the like. An array can be formed from two species of molecule, although it is preferred that the array contain several tens to thousands of species of molecule, preferably from about 50 to about 1000 species. Each species of course can be present in multiple copies if desired.

An "analyte" is a molecule whose detection is desired and which selectively or specifically binds to a molecular probe. An analyte can be the same or different type of molecule as the molecular probe to which it binds.

The steps involved in constructing an array by the process of this invention are diagrammed in Figure 1.

A substantially planar "discrete physical entity" (step a, 1) is derivatized with chemically reactive groups (step b, 3). These groups are

covalently attached to linker molecules (step c, 5). Of course, either or both of these steps can be bypassed if suitable functional groupings and/or linkers are inherent in the material selected for use. The linkers serve as attachment sites for chemical moieties. The linkers are contacted with a solution or droplets of chemical moieties. After binding has taken place between the linkers and chemical moieties, unreacted moieties are removed by washing. Unreacted linkers are treated so as to render them chemically inert in successive array manufacturing steps and minimize their ability to interact with analytes during subsequent assay procedures. This treatment is generically referred to herein as "capping". Thus, e.g., a reactive aldehyde or isothiocyanate group can be capped with an amine or ammonia, a reactive epoxy group can be converted with an acidic solution into a diol, and so on. In step (d), all of the linkers are shown attached to the same species of chemical moiety (7). It should be understood however that more than one species of chemical moiety may be linked to a particular entity as a matter of choice. The material is subdivided into individual tiles (step (e), 9). The subdivision can take place prior to or after step (b). In step (f), tiles comprising the same or different species of chemical moiety (shown generally at 11) are arranged on a support (13) to form an array. In an embodiment of the invention shown in Figure 2, the tiles (20) are cylindrically arranged on a support (22). The support can be a solid rod having tiles disposed on the periphery as shown here, or the support can be a tubular structure wherein the tiles are disposed on the exterior or interior surface of the tube, or between exterior and interior surfaces, if these are spaced apart. Other variations of this shape are intended to be within the scope of this invention.

Any material can be used as a discrete physical entity, provided it is capable of subdivision into tiles, is compatible with the chemistry selected for attachment of chemical moieties to the surface, and compatible optically

with detection method of the assay in which the array is to be used. Examples of suitable materials include, without limitation, glass, silicon, and plastic.

A routine method for derivatizing a glass or silicon surface for attachment of linkers is by formation of siloxane bonds, using organosilanes such as such as 3-glycidoxypropyl- trimethoxysilane ("GOPS"), 3-aminopropyltriethoxysilane (APS), and the like, which have well-understood chemistries. The linker molecule may be a bifunctional reagent that covalently binds the surface to one group and the chemical moiety to the other. Alternatively, the linker may be a reagent that is bound to the surface covalently (e.g., streptavidin) and to the molecule of interest by a high affinity noncovalent interaction(e.g., biotin). Methods for covalently linking chemical moieties to various materials for use in affinity purification procedures are well-known. See, generally, *Affinity Techniques. Enzyme Purification: Part B. Methods in Enzymology*, Vol. 34, ed. W.B. Jakoby, M. Wilchek, Acad. Press, NY (1974) and *Immobilized Biochemicals and Affinity Chromatography, Advances in Experimental Medicine and Biology*, Vol. 42, ed. R. Dunlap, Plenum Press, NY (1974). The covalent attachment of oligonucleotides to solid supports for use in hybridization assays is described in Ghosh & Musso, Nuc.Acids Res.15: 5353-5372 (1987) and Eggers et al, BioTechniques 17: 516-524 (1994). Of course, the attached chemical moieties must able to interact freely with analytes in binding assays (e.g., an attached oligonucleotide must be free to hybridize to a complementary nucleic acid or to bind a sequence-specific protein, an antigen must be capable of interacting with an antibody, and so on).

The chemical moieties intended for use in the arrays of this invention are bioorganic molecules as defined above, having molecular weights in the range of about several hundreds of daltons to about several hundreds of kilodaltons. The density of molecules attached to a single physically

discrete entity is intended to be in the range of about 1000 to about 100,000 molecules per square micron of surface. Various methods can be used to measure the density of molecules on monolayer surfaces. For example, the chemical moiety can be provided with a hydrolyzable group that is cleaved and measured after the moiety has attached to surface, or the chemical moiety may include labels that are directly measurable by spectrometric or microscopic techniques. These techniques and measurements are within the knowledge of one skilled in the art.

The chemical moieties are contained in a solution that can be delivered to the attachment surface of the entity in the form of droplets (see, e.g., EP 0268237 for an example of an apparatus suitable for dispensing and printing reagents) or, preferably, the solution can be held in contact with the surface. It is intended that some of the arrays formed by the process of this invention will comprise multiple arrays of chemical moieties, which can be formed in various ways. For example, an array can be printed onto a tile for placement into a tiled array. Alternatively, multiple stripes of chemical moieties, each stripe containing a unique species of chemical moiety, can be deposited on a discrete physical entity and the entity divided into multiple tiles that include each of the species at a known position on the tiles.

As noted above, the tiles can be formed in any manner appropriate to subdividing the material. Typically, the material is diced with a commercial dicing saw in the following manner. The material is placed on a thin film adhesive backing for mounting on a vacuum chuck. The dicing instrument is programmed with information about the shape of the material to be cut, the desired depth of cutting, and speed of travel of the chuck towards the blade (assuming the position of the blade is fixed). The material is cut in a first direction with a metal or diamond-impregnated blade rotating at a speed of about 20,000 rpm. Debris generated by cutting can be directed away from the cut surface with a jet of air, gas or liquid. The material is then rotated

through a desired angle and cutting is continued in a second direction until the formation of tiles is completed.

The array is formed by transferring the tiles from the thin film adhesive backing (see above) to a support in a stable predetermined spatial arrangement. The transfer (herein referred to as "picking and placing") can be performed with procedures that are known in the manufacture of integrated circuits and LEDs (see, e.g., US Patent No. 5,256,792). The following automated procedure is an example of a robotics procedure that has been used to pick and place tiles comprising oligonucleotides and proteins one at a time on a support in a stable spatial arrangement. An individual tile resting on an adhesive carrier within an x-y grid was located with the aid of a camera. The tile was ejected from the underside of its adhesive backing with a needle, picked up with a vacuum probe, re-inspected with a camera, moved with an x-y planar motor to a predetermined position on a support, and inserted into a holder in the support. Preferably, the tiles are arranged in a circular pattern and held in place by grooved channels formed within the support, rather than by an adhesive. The techniques for forming microstructures such as pockets, grooves or channels capable of attaching tiles in a support are well-known in the art of microfabrication.

The arrays formed by the above-described process are intended for use in a molecular recognition-based assay, in which a sample containing an analyte whose detection is desired is brought into contact with an array of molecules of known structure or activity located at predetermined spatial positions on a support; the analyte is recognized by and selectively binds to an array molecule; and the binding is of sufficiently high affinity to permit the analyte to be retained by the array molecule until detection of the analyte has been accomplished. The selective recognition might be based on a distinguishing physicochemical characteristic of the analyte (e.g., a domain

having a particular charge distribution or polarity that is capable of recognition by an array molecule), or a specific chemical feature of the analyte (e.g., a specific primary sequence in a nucleic acid, protein or polysaccharide, a secondary or higher order conformational structure, or a specific chemical group or combination of groups to form an active site). It is contemplated that the arrays formed by the process of this invention will be useful for screening chemical and molecular biological libraries for new therapeutic agents, for identifying ligands for known biological receptors and new receptors for known ligands, for identifying expressed genes, characterizing genetic polymorphisms, genotyping human populations for diagnostic and therapeutic purposes, and many other uses.

In using an array formed according to the process of this invention, the identity of a chemical moiety bound to an analyte at any particular location in the array can be determined by detecting the location of the analyte and linking this with the array's tagged file. The tagged file is a file of information wherein the identity and position of each chemical moiety in the array pertaining to the file is stored. There are various methods of linking this tagged file with the physical array. For example, the tagged file can be physically encoded on the array or its housing by means of a silicon chip, magnetic strip or bar code. Alternatively, the information identifying the array to a particular tagged file might be included on an array or its housing, with the actual file stored in the data analysis device or in a computer in communication with the device. The linking of the tagged file with the physical array would take place at the time of data analysis. Yet another way of doing this would be to store the tagged file in a device such as a disc or card that could be inserted into the data analysis device by the array user at the time the array was used in the assay.

It should be understood that the above description and examples are intended to illustrate the invention and to provide those of ordinary skill in

the art with a complete disclosure and description of how to use the method of the invention, and is not intended to limit the scope of what the inventors regard as their invention. The process of forming the array can be varied in numerous insubstantial ways including changing the order in which the steps are carried out, the selection of materials used, the geometry of the array, the size and shape of the tiles, the methods of forming discrete physical entities comprising chemical moieties and subdividing these into tiles, and the method of picking, placing and immobilizing tiles on a supporting surface. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains. All of the above-mentioned patents and publications are incorporated by reference herein.

Claims

1. A process for constructing an array of chemical moieties, said process comprising the steps of:
 - (a) forming a multiplicity of tiles, said tiles comprising a substantially planar discrete physical entity having one or more species of chemical moiety attached thereto; and
 - (b) picking and placing said tiles stably on a support at spatially distinct ascertainable locations to form an array of chemical moieties, wherein said formed array comprises at least two species of chemical moiety.
2. The process of Claim 1, wherein said tiles comprise a solid, a gel, a membrane or a combination of these materials, and said tiles are adapted with means for attaching a chemical moiety thereto.
3. The process of Claim 2, wherein said tiles are regularly-shaped and have linear or radial dimensions in the range of about 100 μm to about 10 mm.
4. The process of Claim 3, wherein said tiles are placed stably on a support by insertion into a pocket formed therein.
5. The process of Claim 3, wherein said tiles are placed stably on a support with an adhesive.
6. The process of Claim 3, wherein the chemical moieties in said array comprise bioorganic molecular probes.

7. The process of Claim 6, wherein said probes are contained in a solution and said solution is dispensed as droplets at predetermined locations of said discrete physical entity for the attachment of said chemical moieties thereto.
8. The process of Claim 6, wherein said probes are contained in a solution, and said solution is placed in contact with said discrete physical entity for the attachment of said chemical moieties thereto.
9. The process of Claim 6, wherein said bioorganic molecular probes are selected from the group consisting of nucleic acids or portions thereof, proteins or portions thereof, polysaccharides or portions thereof, and lipids or portions thereof.
10. The process of Claim 9, wherein said array comprises from about 50 to about 1000 species of probes and said probes are attached to said entities at a density of about 1000 to about 100,000 probes per square micron.
11. The process of Claim 9, wherein said bioorganic molecules are nucleic acids and proteins.
12. A process for constructing an array of bioorganic molecular probes for use in a binding assay, said process comprising the steps of:
 - (a) providing a multiplicity of discrete physical entities comprised of a substantially planar subdividable material and provided with sites for the covalent attachment of a bioorganic molecular probe thereto;
 - (b) contacting said material with a molecular probe for sufficient time to attach said probe to said material;

- (c) removing unreacted probe molecules and capping unreacted attachment sites;
- (d) subdividing said material into regularly-shaped tiles having linear or radial dimensions from about 250 μm to about 1000 μm ; and
- (e) picking and placing said tiles stably on a support at a separate and distinct spatial location thereby forming an array of molecular probes, wherein said array comprises from about 50 to about 1000 species of probes.

13. A process for constructing a chemical array substantially as herein described with reference to each of the accompanying drawings.

14. An array formed by the process of any preceding claim.

15. An array of at least two species of chemical moiety formed by the process of Claim 2, comprising:

- (a) a support; and
- (b) a multiplicity of tiles, wherein said tiles are comprised of a substantially planar discrete physical entity having one or more species of chemical moiety attached thereto at spatially distinct ascertainable locations.

16. An array according to Claim 15, further comprising means for encoding the spatial location and identity of every chemical moiety in said array.

17. An array according to Claim 15, wherein said chemical moiety comprises a bioorganic molecular probe selected from the group consisting of nucleic acids or portions thereof, proteins or portions thereof, polysaccharides or portions thereof and lipids or portions of thereof.

18. An array according to Claim 15, wherein said discrete physical entities comprise regularly-shaped tiles having linear or radial dimensions in the range of about 100 μm on a side to about 10 mm on a side.

19. An array according to Claim 18, wherein said discrete physical entities are arranged in a spiral or circular pattern on said support.
20. An array according to Claim 18, wherein said discrete physical entities are arranged in a rectangular pattern on said support.
21. An array according to Claim 18, wherein said discrete physical entities have a cylindrical arrangement on said support.
22. An array according to Claim 18, wherein one or more of said discrete physical entities is comprised of an array of bioorganic molecular probes.
23. An array substantially as herein described with reference to each of the accompanying drawings.



The
Patent
Office
17

Application No: GB 9720959.7
Claims searched: 1-23

Examiner: Dr J Houlihan
Date of search: 27 March 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.P): G1B (BAG)

Int CI (Ed.6): C07K 1/04, 17/00, 17/02, 17/06; C12Q 1/68

Other: ONLINE: WPI, CLAIMS

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	WO 96/18903 A1 (SELECTIDE CORP.) page 15 line 15-page 16 line 12	
X	WO 95/33846 A1 (AFFYMAX TECH.) page 2 lines 30-37; page 6 line 3-page 7 line 14; page 20 lines 23-31; page 22 lines 10-22	1-12 at least
X	WO 94/05394 A1 (ARRIS PHARM. CORP.) page 8 lines 1-18; page 9 lines 18-25; Example 2	1 at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)